consequences for flavour analysis. There will most likely be a development of larger capacity sampling devices using adsorbant tubes and SPME fibres for headspace analysis. The desire to analyse unstable volatiles will lead the analyst to develop derivatization techniques. GC-O will become better known and will not be used only for flavour and fragrance analysis. The evolution of this technique may see the development of expert systems, such as voice recognition software to automatically annotate aromagrams and software to help with interpretation against known chemical data and correlation with sensory data. The development of flavour databases that combine chromatographic and spectroscopic data will continue. Increasing the speed of analysis will see fast GC develop for flavour quality control analysis. An interesting development in the task of comparing data from different instruments with different detectors is retention-time (RT) locking. This has already been successfully applied and will be aided by the development of specific RT lock flavour and fragrance libraries.

See also: **II/Chromatography: Gas:** Derivatization; Detectors: General (Flame Ionization Detectors and Thermal Conductivity Detectors); Detectors: Mass Spectrometry; Detectors: Selective; Headspace Gas Chromatography; Large-Scale Gas Chromatography; Multidimensional Gas Chromatography; **Extraction:** Solid-Phase Microextraction. **III/Allergens in Perfumes: Gas Chromatography-Mass Spectrometry. Chiral Separations:** Gas Chromatography. **Fragrances: Gas Chromatography. Pheromones:** Gas Chromatography. **Solid-Phase Micro-Extraction:** Overview.

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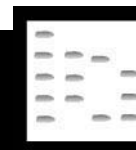
FOAM COUNTERCURRENT CHROMATOGRAPHY



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Introduction

Foam separation methods have long been used for the separation of various samples ranging from metal ions to mineral particles. The separation is based on a unique parameter of foaming capacity or foam affinity of samples in aqueous solution and it has a great potential for application to biological samples. However, the use of this method in research laboratories has been extremely limited, mainly due to a lack of efficient instruments. Foam separation instruments generally consist of a single tubular column where the foam is generated by introducing the gas phase at the bottom of the column (**Figure 1**). Under the gravitational field, the foam moves upwards towards the top of the column to collect foam-active materials. Although various mixing devices such as baffles, solid beads and rotary mixers are used to improve contact, the use of a short column under

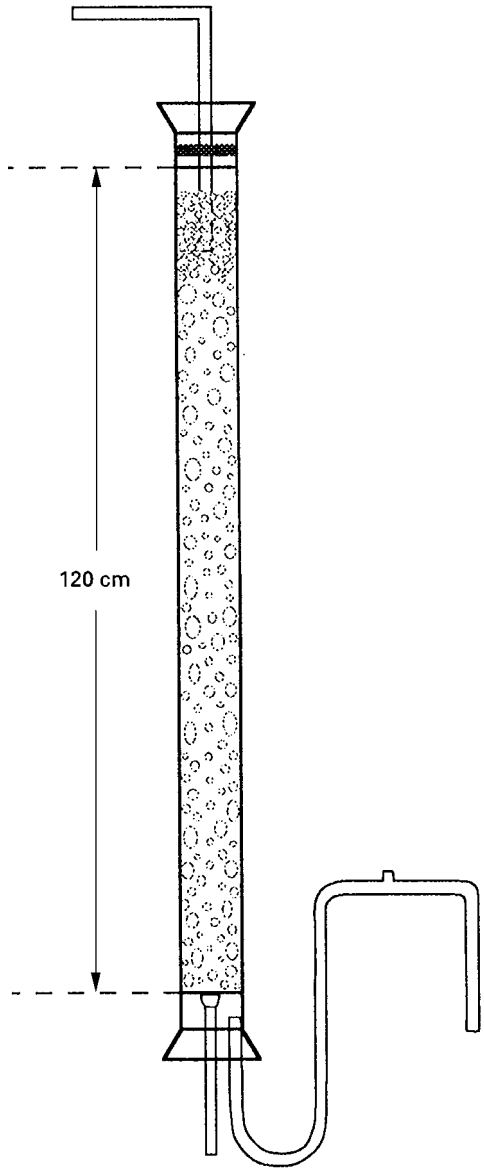

Figure 1 Conventional foam separation column.

a gravitational field limits the efficiency of these systems and consequently, the separation is inefficient.

In 1976, foam countercurrent chromatography (CCC) was developed to improve the foam separation technology. In this foam CCC method, foam and liquid undergo countercurrent movement through a long, fine, Teflon tube (10 m × 2.6 mm i.d.) under a strong centrifugal force field as illustrated in **Figure 2**. This article describes the foam CCC technology and its application to a variety of samples.

Apparatus of Foam CCC

Figure 3 illustrates the design of the foam countercurrent chromatograph. The motor drives the rotary frame around the central axis of the centrifuge. The rotary frame holds a coiled separation column and a counterweight symmetrically at a distance of 20 cm from the central axis of the centrifuge. A set of gears and pulleys produces synchronous planetary motion of the coiled column. This planetary motion induces a countercurrent movement between foam and the mother liquid through a long, narrow, coiled tube. Introduction of a sample mixture into the coil results in the separation of sample components. Foam-active components are quickly carried with the foaming stream and are collected from one end of the coil while the rest move with the liquid stream in the opposite direction and are collected from the other end of the coil (Figure 2).

The column design for foam CCC is shown in **Figure 4**. The coil consists of a 10 m long, 2.6 mm i.d. Teflon tube with a 50 mL capacity. The column is equipped with five flow channels. The liquid is fed from the liquid feed line at the tail and collected from the liquid collection line at the head. Nitrogen gas is fed from the gas feed line at the head and discharged through the foam collection line at the tail while the sample solution is introduced through the sample feed line at the middle of the coil. The head–tail relationship of the rotating coil is conventionally defined by an Archimedean screw force where all objects of different density are driven towards the head. Liquid feed rate and sample injection rate are each separately regulated using needle valves while the foam collection line is left open to the air.

Application

Foam CCC can be applied to a variety of samples having foam affinity. Foam affinity can be classified into two categories: (i) the affinity to the foam-producing carrier; and (ii) the direct affinity to the gas–liquid interface. Samples which lack direct affinity to the gas–liquid interface can be indirectly absorbed to the foam if they have an affinity to the foam-producing agents such as a surfactant. Samples, such as detergents and other foam-producing substances, can be separated without special treatment because they have affinity to the gas–liquid-interface.

Foam Separation Using Surfactants

This technique is applied to the sample having affinity to the foam-producing carrier. Sodium dodecyl sulfate (SDS) and cetyl pyridinium chloride (CPC) were used as carriers to study the effects of electric charges on the foam affinity of various compounds. **Figure 5**

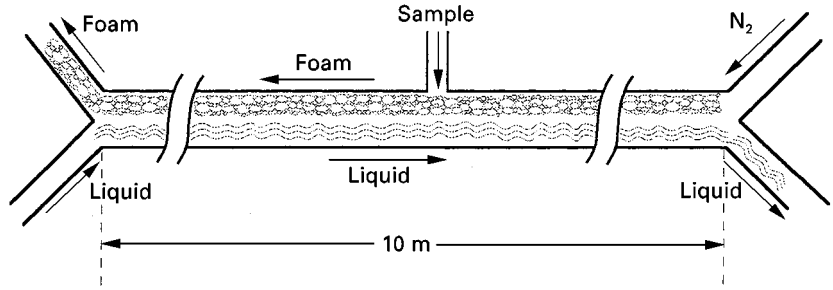

Figure 2 Foam CCC scheme.

illustrates two sets of foam chromatograms obtained from a mixture of methylene blue and DNP–leucine mixture using SDS (top) and CPC (bottom) as carrier reagents. In each chromatogram, the ordinate indicates absorbance values measured at two wavelengths, 430 nm for DNP–leucine and 620 nm for methylene blue. When the sample mixture was introduced with the anionic SDS surfactant, the positively charged methylene blue was adsorbed on to the foam and quickly eluted through the foam collection line (top, right) while the negatively charged DNP–leucine was carried with the liquid stream in

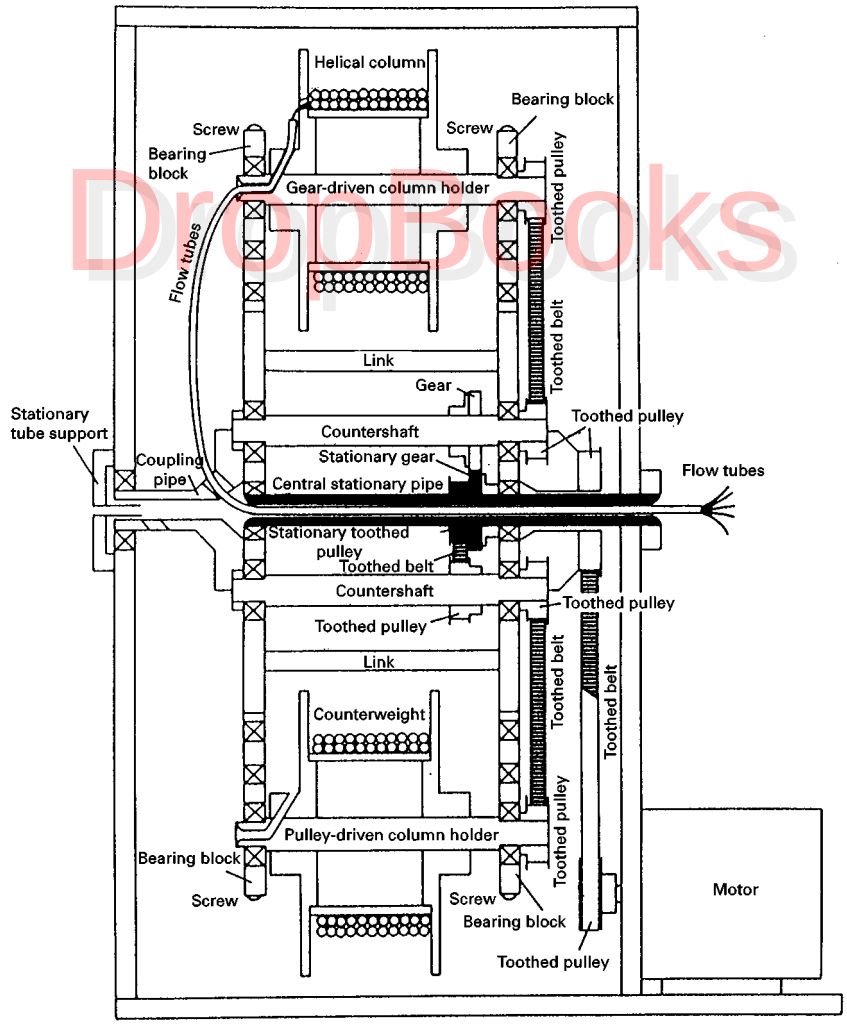

Figure 3 Design of foam CCC centrifuge. (Reproduced from Ito (1985) with permission from Marcel Dekker Inc.)

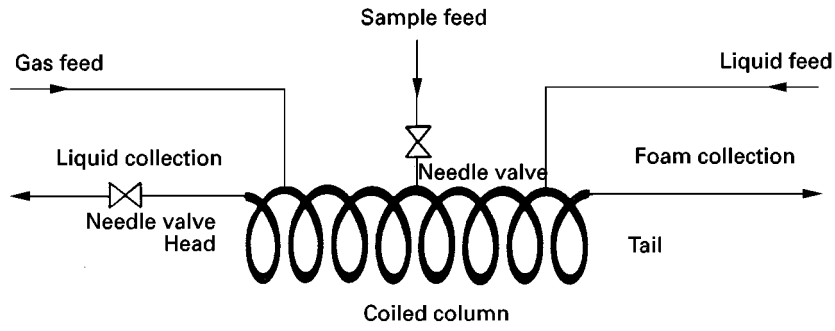

Figure 4 Column design for foam CCC.

the opposite direction and eluted through the liquid collection line (top, left). Similarly, when the same sample mixture was eluted with the cationic CPC surfactant, the negatively charged DNP–leucine was totally eluted through the foam collection line (bottom, right) and positively charged methylene blue through the liquid collection line (bottom, left).

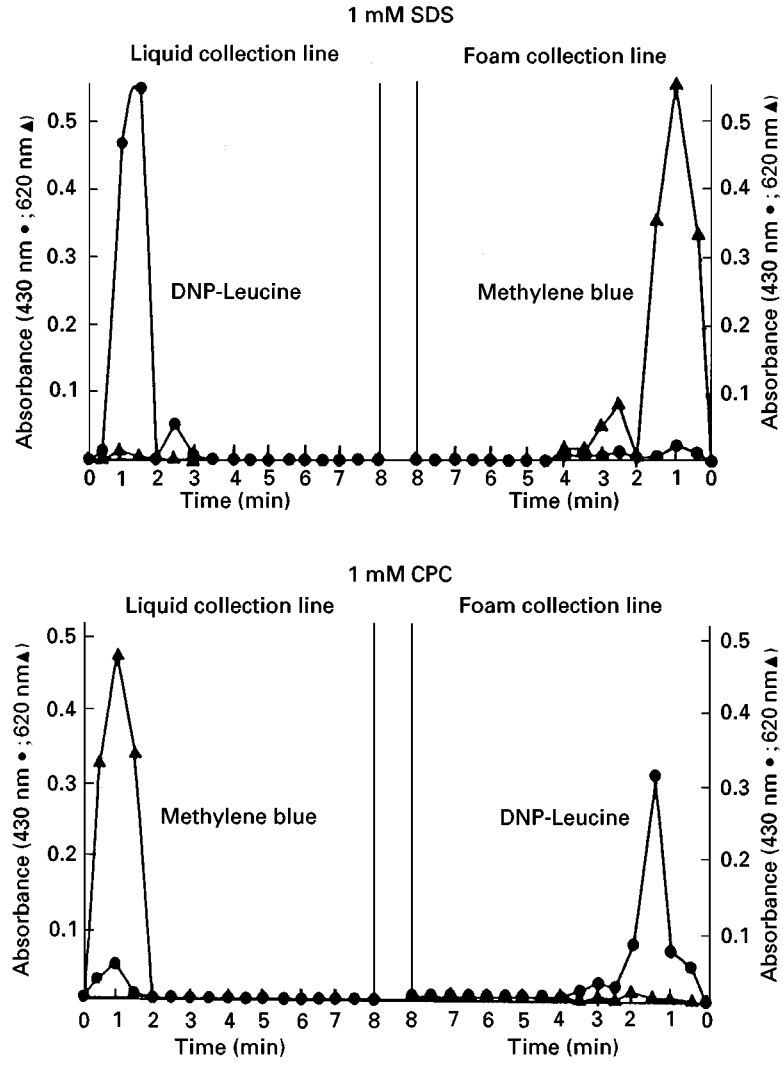

Figure 5 Separation of methylene blue and DNP–leucine by foam CCC. (Reproduced from Bhatnagar and Ito (1988) with permission from Marcel Dekker Inc.)

Foam Separation Without Surfactants

Using ionic surfactant as carriers, the samples which lack direct affinity to the gas–liquid phase interface can be separated if they have opposite electric charges. However, in this case complicated procedures are required to remove the surfactants after fractionation. On the other hand, many natural products have foaming capacity, and therefore foam CCC may be performed without such surfactants.

In order to demonstrate this possibility, bacitracin complex (BC) was selected as a test sample, because it has a strong foaming capacity. Foam CCC for separation and enrichment of BC components has been conducted using nitrogen gas and distilled water entirely free of surfactant or other additives. The following sections describe chromatographic fractionation of BC with batch sample loading and enrichment of foam-active compounds from a bulk liquid on continuous sample feeding.

Batch sample loading BC is a basic cyclic peptide antibiotic commonly used as a feed additive for livestock worldwide. It consists of more than 20 components, but chemical structures of these components are still unknown except for BCs-A and -F.

Foam separation of BC components was initiated by simultaneous introduction of distilled water from the tail and nitrogen gas from the head into the rotating column while the needle valve at the liquid collection line was fully opened. After a steady-state hydrodynamic equilibrium was reached, the pump was stopped and the sample solution was injected through the sample port. After the desired standing time, the needle valve opening was adjusted to the desired level and pumping was started again. Effluents from both outlets were collected at 15 second intervals.

Figure 6 shows the elution curve of BC components from the foam outlet. The vertical axis indicates the absorbance at 234 nm and the horizontal line, the fraction number. This elution curve shows three major peaks as indicated by arrows. The fractions corresponding to these peaks were subjected to HPLC analysis.

HPLC chromatograms of BC components in the foam fractions are shown in **Figure 7**. Under reversed-phase HPLC conditions, BC was separated into more than fifteen peaks. Generally, hydrophilic compounds elute earlier than hydrophobic compounds under these conditions. In this study, the HPLC elution time is used to indicate the polarity of the BC components. Thus, BC-A which elutes earlier in HPLC is more hydrophilic than BC-F. The most hydrophobic compounds (peaks 14 and 15) with the longest retention time in HPLC analysis were collected in the first foam fraction with a small amount of less hydrophobic compounds (peaks 11 and 13). Peak 15 is hardly visible in the HPLC chromatogram of the original sample due to its low concentration, but the same peak is clearly observed in the chromatogram of the first foam fraction. BC-A was almost completely isolated in peak 11 from other components eluted in the tenth fraction. In the twentieth fraction, peak 7 appeared in the HPLC chromatogram. Components with lower hydrophobicity than peak 7 did not appear in the foam fractions. These results clearly indicate that the bacitracin components are separated in the order of hydrophobicity of the molecule in the foam fractions with the most hydrophobic compounds being eluted first.

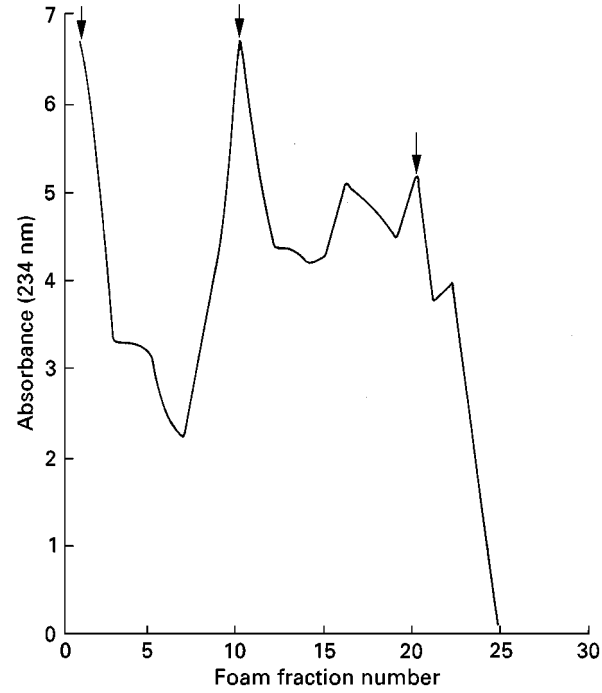

Figure 6 Elution curve of bacitracin components from foam line. Foam CCC conditions: liquid flow rate, 3.2 mL min^{-1}; needle valve, 0.8 turn open; standing time ater sample injection, 5 min; N_2 gas pressure, 80 psi; revolution speed, 500 rpm; sample size, 5 mg per 0.5 mL in H_2O; fractionation, 15 s per tube.

As described above, BC components were separated according to their hydrophobicity using foam CCC without surfactant. This method can also be applied to continuous sample feeding as described below.

Continuous sample feeding On the basis of the preliminary experimental results, the following foam CCC conditions were chosen for large scale sample feeding. The conditions were as follows: needle valve, 2.0 turns open; sample concentration, 50 p.p.m.; sample size, 2.5 L; sample feed rate, 1.5 mL min^{-1} at 40 psi; ni-

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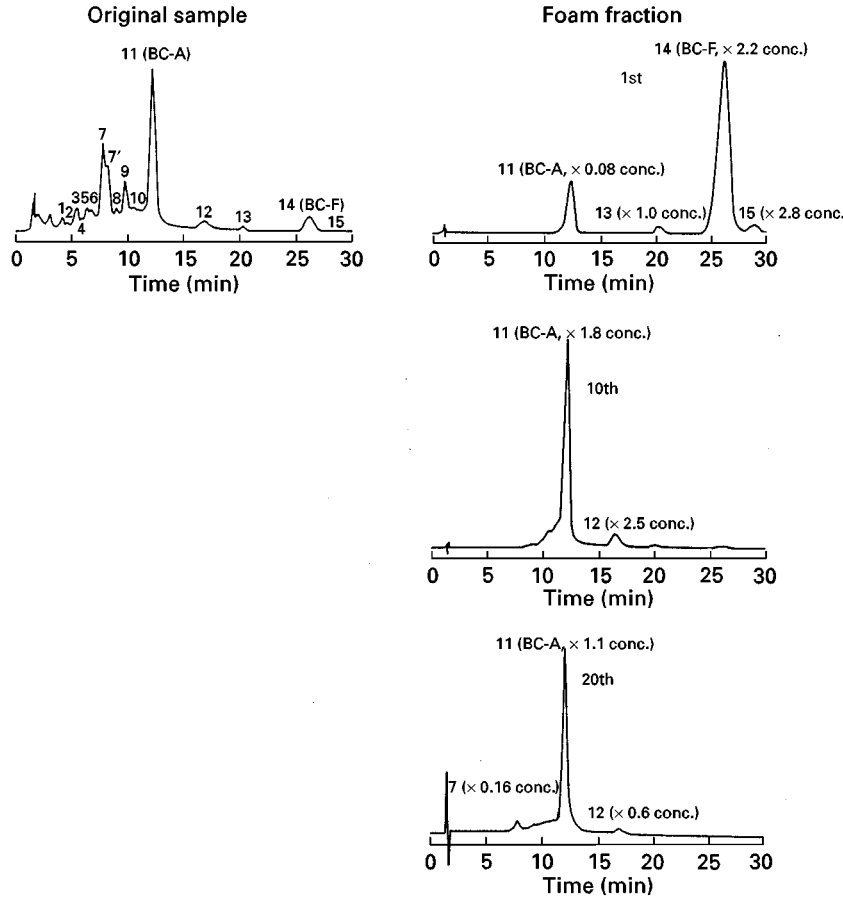

Figure 7 High performance liquid chromatographic analyses of bacitracin components in foam fractions. HPLC conditions: column, Capcell Pak C18 (5 μm, 150 × 4.6 mm, i.d.); mobile phase, CH_3OH/0.04 M Na_2HPO_4 (62/38); flow rate: 1 mL min^{-1}; detection, 234 nm.

trogen gas feed pressure, 80 psi; liquid flow rate, 0; sample collection, pooling the foam and the liquid effluents separately; and revolution speed, 500 rpm.

Figure 8 shows the results of HPLC analyses of bacitracin in the foam and liquid fractions obtained by large scale continuous foam CCC. The concentration in the foam fraction increases with the hydrophobicity of the components. Peak 3 was enriched 22 times; peak 7, 31 times; peak 11, 1400 times; peak 12, 1070 times; peak 13, 1380 times; and peak 14, 2260 times. In the liquid fraction, peaks 3 and 7 were barely detected. Thus, continuous enrichment and concentration in foam CCC is quite effective for the detection and isolation of a small amount of natural product with a foaming capacity.

Estimation of applicability of the sample to foam CCC In order to apply the foam CCC technique to various natural products, it is necessary to establish a set of physicochemical parameters which reliably indicate their suitability for foam CCC. In foam CCC the sample solution is introduced from the sample inlet at the middle of the coiled column where it is immediately mixed with the N_2 stream and the generated foam moves towards the foam outlet at the tail. Since the coiled column consists of a 10-m-long tube, the foam must travel through a 5-m-long narrow coiled path before it reaches the foam outlet. As the foam travels through the decreasing pressure gradient along the coil, every bubble is expanded while the excess fluid is removed by the centrifugal force. Therefore, we assume that the foam must be subjected to a repetitive process of coalescence, eruption and regeneration before reaching the foam outlet at the tail. Consequently, successful foam CCC using nitrogen gas and distilled water free of surfactant requires strong foam-producing capability and foam stability of analytes. A lack of either property would result in its failure.

For this purpose, two parameters were selected, i.e. 'foaming power' and 'foam stability', which can be determined by one simple test. In each test, the sample solution (20 mL) is delivered into a 100 mL graduated cylinder with a ground stopper and the cylinder vigorously shaken for 10 s. The foaming power is expressed by the volume ratio of the result-

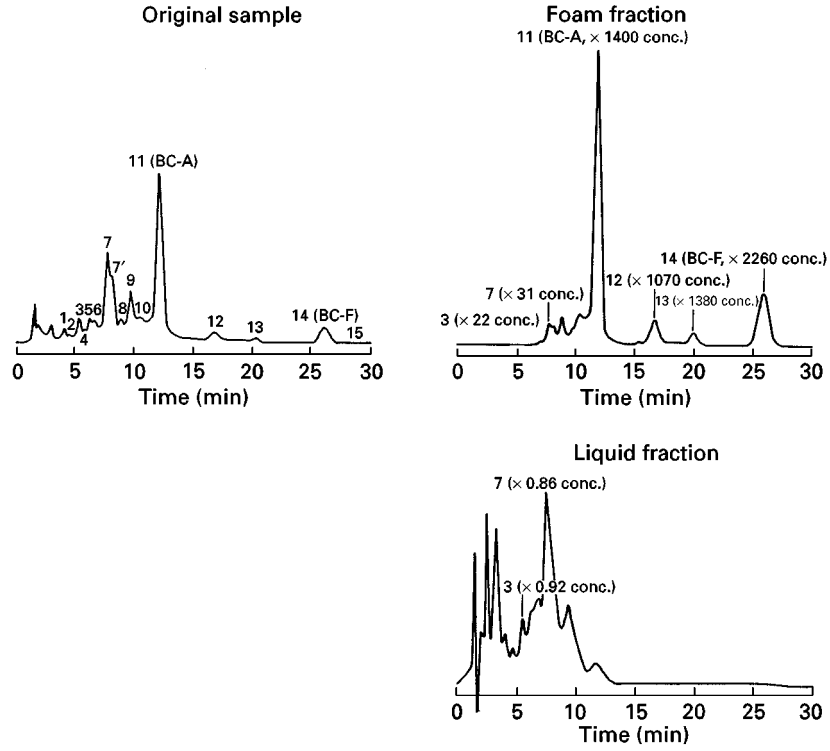

Figure 8 High performance liquid chromatographic analyses of bacitracin components in foam and liquid fractions. HPLC conditions: column, Capcell Pak C18 (5 μm, 150 × 4.6 mm, i.d.); mobile phase, CH_3OH/0.04 M Na_2HPO_4 (62/38); flow rate, 1 mL min^{-1}; detection, 234 nm.

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ing foam to the remaining solution, and the foam stability by the duration of the foam.

In order to correlate the foaming parameters measured by this simple test method to the productivity in foam CCC, the following five samples were selected because of their strong foaming capacities: bacitracin, gardenia yellow, rose bengal, phloxine B, and senega methanol extract. The results of our studies indicated that a sample having a foaming power greater than 1.0 and a foam stability over 250 min could be effectively enriched by foam CCC. These minimum requirements of foaming parameters tentatively determined by the bacitracin experiment were found to be consistent with those obtained from the other four samples.

The above simple test has been applied to enrichment of microcystins, hepatotoxic cyclic peptides produced by cyanobacteria. Microcystins was extracted from the bloom sample 917S with distilled water to obtain two extracts with different foaming capacities. The first extract had foaming power of 1.88 and

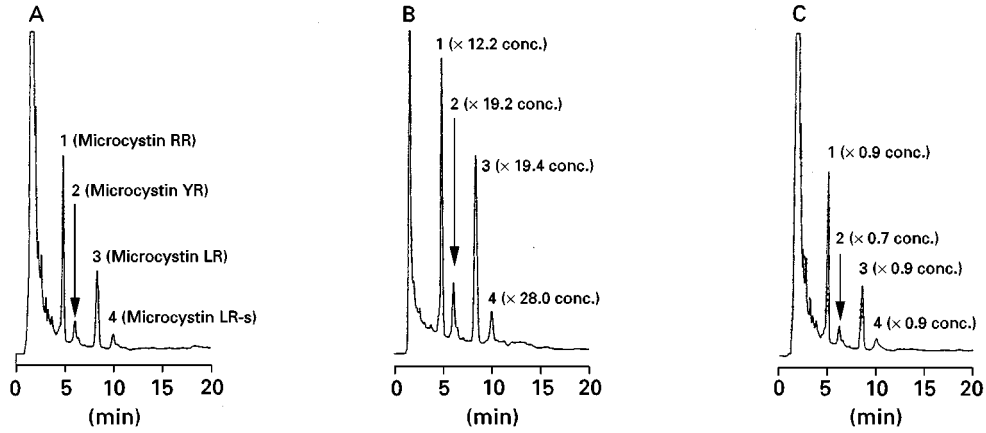

Figure 9 High performance liquid chromatographic analyses of bloom sample 917S extract that satisfies the foaming parameters: A, original sample; B, foam fraction; C, liquid fraction.

foam stability of 93 min which satisfied only the former requirement. The second extract had a foaming power of 1.32 and the foam stability of 720 min which satisfied both requirements for foam CCC. Then, both samples were subjected to foam CCC.

Figure 9A shows a typical HPLC chromatogram of an extract from cyanobacteria bloom sample 917S containing microcystins. As indicated in the chromatogram, peaks 1 (microcystin RR), 2 (microcystin YR), 3 (microcystin LR), and 4 (microcystin LR-s) were chosen to evaluate their foam enrichment. In the first extract, the enriched concentrations of the components are only 3–4 times and polar components with retention times shorter than that of microcystin RR were still present in the foam fraction. The HPLC analysis of foam fraction and liquid fraction of the second extract is shown in **Figures 9**B and C. The enrichment reached 10–30 times and polar components are eliminated from the foam fraction indicating that the target compounds are selectively enriched. The HPLC analysis of liquid fraction of both extracts showed similar profiles. These results indicate that these foaming parameters can be effectively applied to a crude mixture containing a large amount of impurities.

Conclusions

Foam CCC can be successfully applied to a variety of samples having foam affinity with or without surfactants. The present method offers important advantages over the conventional foam separation methods by allowing efficient chromatographic separation of sample in both batch loading and continuous feeding. We believe that the foam CCC technique has a great potential in enrichment, stripping and isolation of foam-active components from various natural and synthetic products in both research laboratories and industrial plants.

See also: **II/Chromatography: Liquid:** Countercurrent Liquid Chromatography. **III/Antibiotics:** High-Speed Countercurrent Chromatography; Liquid Chromatography; Supercritical Fluid Chromatography.

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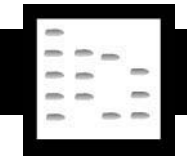
FOOD ADDITIVES



Liquid Chromatography

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Introduction

Food is a complex heterogeneous mixture of a wide range of chemical constituents such as moisture, carbohydrates, proteins, fibres, vitamins, etc. Besides these, processed foods contain a wide array of additives and contaminants. Analysis of product composition is a prerequisite for ascertaining product quality,